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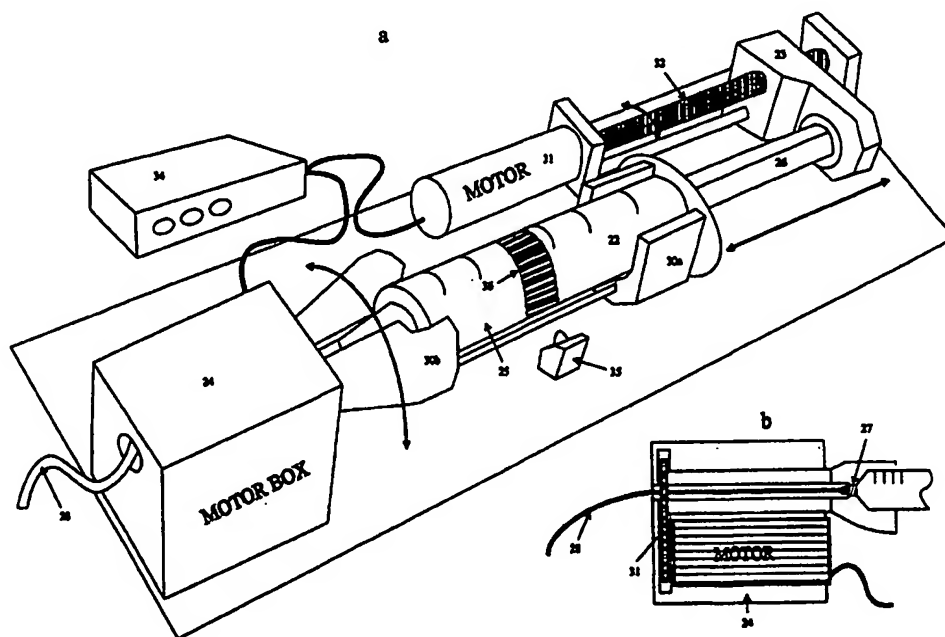
(57) Abstract

A power assisted method and injector device for controllably delivering to patients a dispersion medicament or diagnostically active agent, the homogeneity of which is preserved throughout delivery by agitation thus preventing segregation of particles by gravity or buoyancy. Diagnostically active agents disclosed are gas microbubble suspensions useful in ultrasonic diagnostic imaging and liposomal formulations in which liposome vesicles are loaded with iodinated compounds.

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AUTOMATIC LIQUID INJECTION SYSTEM AND METHOD

Field of the invention

The present invention concerns the administration by
5   injection to patients of liquid compositions for
therapeutic or diagnostic purposes. It more particularly
concerns a power assisted method and device for
controllably dispensing a liquid medicament or
diagnostically active contrast agent, the homogeneity of
10   which is preserved throughout delivery. Typically, the
contrast agent is an aqueous suspension of gas filled
microvesicles, namely microbubbles bounded by a surfactant
stabilized gas/liquid interface, or microballoons bounded
by a tangible material envelope.
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Background Art

Power injectors and mechanically assisted infusion
systems for controllably dispensing therapeutically active
medications are well known in the art. Typically, such
20   devices include an automatic injector for syringes
containing an injectable liquid and a plunger or piston
movable within the barrel of the syringe to expel said
liquid through a tip thereof and injecting into a patient
via a tubing connected to an injecting needle or catheter.
25   For controlling the injections parameters, the plunger is
driven by means of an electromechanical arrangement
organised to push the plunger at a desired rate,
continuously or at chosen intervals, so that the amount of
medication is delivered to the patient's body under
30   strictly determined conditions. For instance, in the case
of intravenous dispensing contrast agent formulations for
diagnostic purposes (X-ray, MRI or ultrasound), the rate
and the mode of injection can be accurately controlled to
match the requirements of the imaging methods and detector
35   systems used to investigate the circulation or a specific
organ in the body. Typical automated injection devices are
illustrated and described in US-5,176,646 incorporated
herein by reference.

Although the automated injectors known are highly
40   sophisticated instruments capable of mastering most

injection problems experienced in practice, there remains
at least one variable factor not yet under control. Indeed
the known power injectors have no control of the
homogeneity of the liquid stored within the syringe barrel
5   during the course of its application. This kind of problem
is of course non-existent with "true solutions" (i.e.
solutions to the molecular level) since in this case no
concentration change can occur in the course of time; it
however may become important when the injectable
10   formulation is a suspension or dispersion of active
particles which tend to settle, coalesce or segregate with
time in the syringe. Indeed, even some modest separation of
the particles by gravity or otherwise from the carrier
liquid in the course of administration of the formulation
15   may have very important influence on reproducibility and
reliability of the tests. Hence, in this case, a method and
means to keep the syringe content homogeneous during
injection is highly desirable. The present method and
device constitute a very effective solution to the
20   aforediscussed problem.

Summary of the invention

Briefly stated, in order to secure homogeneity of a
liquid suspension of particles within the barrel of an
25   injector device, the invention provides a method and means
whereby the particles are kept under sufficient agitation
so as not to settle, segregate or agglomerate in the
carrier liquid. This may involve acting on the carrier
liquid itself, i.e. on the bulk of the suspension, or may
30   involve acting only on the particles (in this case, one
would expect the moving particles to impart motion to the
carrier liquid by viscous friction). The agitation means
may be provided within the syringe or in some cases outside
thereof; for instance with magnetic particles, the
35   particles can be subjected to an external variable magnetic
field, the oscillation or rotation of which will set them
into motion, the moving particles then acting on the
carrier liquid and keeping the suspension homogeneous.

In the case of particles not sensitive to external fields, mechanical agitation is provided to the extent that it is sufficient to keep the suspension homogeneous but insufficient to break or damage the particles or disturb their distribution. For this, the syringe barrel is subjected to motion, said motion being continuous or discontinuous, regular or irregular; the motion can possibly have a shaking, rocking or oscillating effect on the syringe. The frequency, intensity and rate of the motion is such that it will not interfere with the control of delivery parameters of the suspension.

The embodiments disclosed below in connection with the annexed drawings provides very effective means to keep the syringe content under sufficient agitation to secure injection of a homogeneous therapeutic or diagnostic liquid compositions into a patient.

Brief description of the drawings

Fig. 1 is a schematic view in perspective of a device for agitating a liquid within the syringe of a power driven automatic injector system of the invention.

Fig. 2 is a graph illustrating the homogeneity variations in a suspension of microbubbles contained in a syringe, the latter being either still or subjected to motion according to the invention.

Fig. 3 is a graph illustrating the gas volume and in vitro intensity of samples with and without treatment according to the invention.

Fig. 4a is a schematic view in perspective of another device for agitating a liquid within the syringe of a power driven automatic injector system of the invention. In this embodiment, the syringe is held by a supporting bracket, the latter being driven into motion by a motor.

Fig 4b is a schematical sectional view of the motor driving means of the embodiment of Fig 4a.

Detailed description of the invention

The device represented schematically in Fig. 1
comprises a series of co-operating elements mounted on a
board 1. Such schematic representation of the present
device is only for clarity and better understanding of the
device's operation. Obviously, in its actual commercial
construction, the device is in the form of a much more
compact and sophisticated apparatus, for instance in the
form of an instrument like the *Perfusor® fm* of the Firm
BRAUN Meslungen AG, D-34209, Meslungen, Germany (displayed
in Publication B.03.01.95 No 0879 0744), or like the
apparatuses disclosed in US-A-4,652,260 and US-A-5,176,502,
both being incorporated herein by reference.

The present device comprises the following working
components: a syringe 2 shown in an uplifted position, an
automatic power driving unit 3 for acting on the syringe, a
pair of syringe motioning units 4 for liquid agitation, and
a control box 14 for controlling operation of the units 4.

The syringe 2 has a barrel 5, a plunger 6 sliding in
the barrel and a tip connector 7 linked to a tubing 8, the
latter leading to an injection needle 9. The needle 9 is
for injecting an administrable liquid into the tissues or
the circulation of a patient.

The power driving unit 3 has an electromechanically
controlled pusher rod 10 for acting on the rear end 11 of
the syringe plunger, and a control knob 12 for setting the
automatic driving parameters that will marshal the action
of the rod 10.

Each unit 4 is equipped with two rollers 13,
themselves driven into rotation by electric motors within
the units and not represented in the drawing. The rotation
of the rollers 13 is governed by means of a box 14 via lead
wires 15 connected to said motors.

In operation, an injectable carrier liquid with
particles (e.g. gas-filled microballoons) in suspension is
introduced into the barrel 5 of the syringe 2 through the
tip 7, this being consecutive to the retraction (manual or
mechanical) of the plunger 6, so that an adequate pumping

action is provided. Then the syringe is placed on the
rollers 13, so that the flange 16 thereof abuts the
roller's edge 17, this being for retaining the syringe in
its relative position against unwanted longitudinal
translation. In this situation, the pushing rod 10 of the
driving unit 3 couples with the plunger's end 11, so that
any forward displacement of the rod 10 is transferred to
the plunger with consequent expelling of the liquid toward
the needle 9 for injection.

During injection, the rollers will alternately rotate
the syringe a certain angle in one direction, say 30°, 60°,
90°, 180°, 270° or 360° and then, reciprocably, in the
opposite direction. This balancing motion, which may be
carried out in a stepwise manner, will move the liquid
carrier to such an extent that any separation or
segregation of the particles is hindered. This is very
efficient for instance in the case of suspensions of gas-
filled microbubbles used in echography since there is
always a bubble size distribution in such suspensions, the
larger bubbles tending to rise faster than the smaller ones
by buoyancy. In a variant, the syringe can be made to
rotate in one direction only, provided that the connector
tip 7 thereof is made to freely rotate in order to prevent
distortion of the tubing 8. Normally, the rate of rotation
impressed by the rollers 13 is from about 0.5 to 200 rpm
depending upon the suspension viscosity. This rate should
be sufficient to keep the particles in homogeneous
suspension but insufficient to break the particles or
disturb their distribution in the carrier liquid. If
necessary, in the case of more viscous suspensions, an
additional vibrational motion of a few Hz to a few hundreds
of Hz can be applied to the syringe by means of a pitch-
fork or pitch-pipe. It should be mentioned that at very
high rotation rates (e.g. 1,000 rpm or more) the radial
speed may become dominant which will result in axial
concentration of the microbubbles in the middle of the
syringe. Rotational speeds at which the radial component
becomes important are to be avoided as under such

conditions the suspension will become non-homogeneous again. This is clearly undesired.

In a variant, the unit 4 can have the form of a closable housing equipped with fixed syringe retaining means, i.e. other than the rollers edges 17 and, possibly if required, pressure resisting means (like a pressure mantle or jacket) in case the suspension is viscous and exerts undue pressure efforts to the syringe barrel. Also the syringe components can be made of moulded plastic (disposable syringes) and the barrel external surface provided with an integrally moulded relief pattern mating with corresponding pattern on the roller's surface, so that positive grip drive of the syringe is ensured.

Also, the rod 10 and the plunger 6 can be made integral with each other so that filling of the syringe can be controlled by the power unit 3, the pumping action then resulting from a backward displacement of rod 10.

The power unit per se is standard and its nature and operation well known to the skilled person. Embodiments thereof are disclosed in the cited references and also in US-A-5,456,670. The power unit usually contains an electrically powered and controlled helical screw means for mechanically advancing or retracting rod 10 continuously or intermittently, so that the liquid in the syringe can be dispensed continuously or by increments. The various parameters ruling said motions of the syringe piston can be monitored and adjusted by the control 12 and possible other control means not represented in the drawing. Means of unit 3 also ensure that such delivery parameters can be monitored and recorded for display. An instant stop switch (not shown) may also exist, in case the operation of the system should be suddenly interrupted due to a problem with the patient or otherwise.

It should be incidentally noted that although the present embodiment involves rocking the syringe only, one may also consider a modification involving a back and forth rotation of the pumping ensemble, this being achieved by

well known mechanical means adapted to support said pumping
ensemble and to impart motion thereto.

Furthermore, although the present embodiment involves
motion around the longitudinal axis, a variant may include

5   rocking the syringe about a transversal axis.

A second device embodiment illustrated schematically
in Fig 4a and 4b comprises a syringe 22 with a a barrel 25
supported in a rotatable fashion by a bracket 30a-30b and a
plunger 26 sliding in the barrel whose displacement therein

10   is controlled by a a power driven unit 23 capable of moving
forward and backward in engagement with the back pusher end
of the plunger 26. The device also comprises a motor driven
unit 24 encompassing a portion 30b of the supporting
bracket, the latter being rotated through gears 31, as

15   better shown on Fig 4b, for agitation of a liquid
suspension in the syringe barrel. The longitudinal forward
or backward displacement of the unit 23 (acting on the
plunger 26) is effected via a motor 31 which rotates a
screw-bar 32, the latter engaging with a matching threaded

20   portion (not shown) within the unit 23. The device further
comprises an electronically computerized control box 34 for
controlling operation of the units 23 (via motor 31) and
24, and for processing the signals from a laser detector 35
designed to read an identifying mark 36 on the syringe;

25   this mark is for preventing errors in the selection of the
syringe, especially if the syringe is of the prefilled
type. The code of the mark can be according to standard bar
codes. Note in this regard that since the syringe barrel is
set into rotation in the present device, one can use a

30   fixed detector instead of a mobile one which is
advantageous designwise. By counting and recording via box
24 the number of turns of the screw bar 32, the position of
the unit 23 (and consequently of the plunger 26) can be
monitored and regulated at will. The control box 34 can of

35   course comprise further monitoring and visualizing means
(not shown) to optically display and appropriately regulate
the various parameters involved in operation of the device.
As in the previous embodiment, the syringe has a tip 27

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for connecting to a liquid dispensing tubing 28, the latter leading to means for injecting an administrable liquid into a patient.

The operation of the present device is very similar to that of the earlier embodiment and hence needs not be discussed further at length. Suffice to say that it may also comprise security means intended to automatically interrupt the operation in case troubles develop with the patient or otherwise during injection. For instance, the pressure in the syringe barrel can be monitored by registering the force required to push the plunger, this being via the power absorbed by the driving motor 31. A sudden surge, for instance a rapid increase of current in said motor can trigger via the control unit 34 an emergency stop of the device. Alternatively, this effect could also be detected according to usual means by a strain gauge installed in the drive 23.

As already said, the particles of the suspensions in this invention may be of various kinds and involve for instance microspheres containing entrapped air or other gases used in echography. These microspheres may be bounded by a liquid/gas interface (microbubbles), or they may have a tangible membrane envelope of for instance synthetic polylactides or natural polymer like denatured protein such as albumin (microballoons). The carrier liquid for the microbubble suspensions comprises surfactants, preferably saturated phospholipids in laminar or lamellar form such as diacylphosphatidyl derivatives in which the acyl group is a C_{16} or higher fatty acid residue.

The gases used in the microbubbles or microballoons are pure gases or gas mixtures including at least one physiologically acceptable halogenated gas. This halogenated gas is preferably selected among CF_4, C_2F_6, C_3F_8, C_4F_8, C_4F_{10}, C_5F_{12}, C_6F_{14} or SF_6. The gas mixtures can also contain gases such as air, oxygen, nitrogen, helium, xenon or carbon dioxide. In fact in a number of cases microbubbles or microballoons will contain mixtures of

nitrogen or air with at least one perfluorinated gas in proportions which may vary between 1 and 99%.

In the microballoons the membrane is made from a biodegradable material such as biodegradable polymers,
5   solid triglycerides or proteins and are preferably selected from the polymers of polylactic or polyglycolic acid and their copolymers, denatured serum albumin, denatured haemoglobin, lower alkyl polycyanoacrylates, and esters of polyglutamic and polyaspartic acid, tripalmitin or
10   tristearin, etc. In an embodiment, the microballoons are filled with C_3F_8 and the material envelope is made of albumin.

Homogeneity of suspensions of microballoons whose membrane is made of saturated triglycerides such as
15   tripalmitin, trimyristin or tristearin and their mixtures with other tri- or di- glycerides, fatty acids or polymers is particularly interesting as those are used for delivering active ingredients to specific sites within the body. Homogeneity of suspensions of such microballoons has
20   been effectively maintained using the method and the device of the invention.

Other particles whose density is different from that of the carrier liquid may include liposomes filled with iodinated X-ray opacifiers such as iomeprol,
25   iopamidol, iopentol, iohexol, metrizamide, iopromide, iogulamide, iosimide or ioversol or, for instance, coated and uncoated magnetic particles which tend to precipitate in saline or other carriers.

The present injector system can be used in
30   imaging organs, blood vessels and tissues of mammalians, e.g. the ultrasonic imaging of the heart, the liver or spleen, the brain, the kidneys, the blood vessels, etc.

The invention is further illustrated by the following Examples:
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Example 1

A solution of gas filled microbubbles stabilised by a phospholipids interface was prepared according to

Example 1 of US 5,445,813. The dry matter concentration was 5 mg/ml in a saline solution (0.9% NaCl). Typically, the bubble size distribution extended from 0.2 to 15 μm. The concentration of bubbles between 2 and 5 μm was 5×10^7

5   microbubble/ml.

The solution was transferred in a 50 ml plastic syringe and samples were taken in time intervals for analysis. This represent the starting 100% of the bubble concentration. The syringe was mounted in the infusion unit

10 and the elution started. The elution flow was fixed at 1.6 ml/min.

Aliquots of the eluted solution were analysed by Coulter measurement (bubbles distribution; size and concentration) and imaging.

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Table 1

Radius	Va	Radius	Va	Radius	Va	Radius	Va
1.0	0.131	4.5	2.648	8.0	8.368	11.5	17.291
1.5	0.294	5.0	3.269	8.5	9.446	12.0	18.828
2.0	0.523	5.5	3.955	9.0	10.590	12.5	20.429
2.5	0.817	6.0	4.707	9.5	11.800	13.0	22.096
3.0	1.177	6.5	5.524	10.0	13.075	13.5	23.829
3.5	1.602	7.0	6.407	10.5	14.415	14.0	25.626
4.0	2.092	7.5	7.355	11.0	15.820	14.5	27.489

In water, the rate of rise (Va) by buoyancy of air

20 filled microbubbles of radius (a) can be obtained from the following Stokes relation $Va = \frac{2gr}{9h} \times a^2$ where g is the gravitation constant ($9.81 \ ms^{-2}$), r is the density of water (1000 g/l) and h is the viscosity (10^{-3} Kg[s·m]). Table 1 shows a range of such rates (in mm/min) in function to the

25 bubble radius in μm. The tangential speed ($V_r = 2\pi nR$) of a syringe barrel of 28 mm diameter (R = 14 mm) in function to the rotation rpm (n) is given in the next Table 2.

Table 2

n (rpm)	V_r (mm/min)
0.5	2539
1	5278
2	10556
3	15834
4	21112
5	26389
10	52779

It is seen from the foregoing figures that in the
5   case of a suspension of microbubbles of size in the range
of 1-10 μm, very low rates of rotation of the syringe are
sufficient to prevent segregation of the bubbles by
buoyancy. This means that even at low rates of rotation the
tangential speed of the microbubbles in suspension is much
10   larger than buoyancy and that the microbubbles will move
together with the rotating liquid and will not rise to the
top of the syringe.

In a comparative study, the syringe was rotated
along its axis in an alternative mode at a speed of 60 rpm.
15   The results were compared with an experiment where the
syringe was not rotated (under otherwise same experimental
conditions).

Fig. 2 shows the evolution of the concentration
of the total microbubble population and, separately,
20   microbubbles above 8 μm along the elution while Fig. 3
shows the evolution of imaging intensity and the total
bubble volume in the course of elution. In the case of no-
agitation, the concentration decreases rapidly due to
decantation. At the end of the infusion, the concentration
25   rises sharply (not shown) because all the bubbles
accumulate in the upper part of the barrel.

When the syringe is rotated, the bubble
concentration remains constant throughout the entire
infusion.

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The same type of experiments were carried out
under different experimental conditions including different
microbubbles sizes and concentrations, different elution
rates, different rotation types and speed, different
5   syringe types and different particles such as heavy
magnetite particles or other microbubble structures
including phospholipid, tripalmitin or albumin encapsulated
microbubbles. All experiments invariably showed that the
method of infusion disclosed delivers homogeneous
10   suspensions of active agents.

Example 2
Preparation of contrast agents for infusion

15   To test the efficiency of the present invention
(a system of rotary syringe pump), different contrast
agents for ultrasound echography were prepared.

•   Microbubble suspensions

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Phospholipid stabilised microbubbles were obtained in
the following manner. 500 mg DAPC and 50 mg DPPA (Avanti
Polar Lipids, Inc.) were dissolved in hexane/iso-propanol
8/2 (v/v) and dried in a round-bottomed flask using a
25   rotary evaporator and, further, in a vacuum dessicator.
After addition of water (100 ml), the suspension of lipids
was heated at 75°C for 1 hour under agitation and then
extruded through a 0.8 μm polycarbonate filter
(Nuclepore®). The resulting suspension and 10 g of poly-
30   ethyleneglycol (M_w4000) were mixed and lyophilised. 2 g of
the lyophilisate was introduced into a glass vial and
sealed under SF_6 or an air/C_4F_{10} mixture. After
reconstitution with 25 ml NaCl 0.9%, the resulting
suspensions contained about 6×10^8 (SF_6) or 1×10^9 (C_4F_{10})
35   bubbles per ml with a mean diameter in number of 2 μm
(Coulter Multisizer).

• <u>Microballoon suspensions</u>

Gas filled albumin microspheres were prepared as described by Porter T. R. (*J. Am. Coll. Cardio.* **23** (1994) 1440 and PCT/WO 96/38180). 16 ml of human serum albumin (HSA) diluted 1:3 with dextrose (5%) was introduced into a 20 ml syringe and sonicated (sonifier 250 Branson) for 80 seconds in the presence of a flux of C_3F_8 gas (octafluoropropane) at liquid/air interface. The sonicator tip was immersed at about 1 cm below the surface of the solution, the ultrasound energy level was set at output -40 and the temperature of the solution was monitored at 75°C. After removing the foam phase by decantation, the final suspension contained 8×10^8 gas microspheres per millilitre with a mean diameter in number of 2 µm (9 µm in volume) determined by Coulter®. The suspensions are stored at 4°C until use.

<u>Example 3</u>
<u>Determination of the limit of rotation rate for the syringe used for infusion</u>

The effect of syringe rotation on stability of gas microbubble suspensions in the syringe used for infusion has been tested using a 50 ml syringe which was mounted on a rotation system which allows very low rotation speeds (about 1 rpm). Prior to its mounting the syringe was filled with 30 ml of phospholipid stabilised microbubble suspension. The mounted syringe was then rotated at different speeds: 0 (no rotation) 1.3, 2 and 60 rpm (1 Hz) and the suspension monitored taking one sample every 5 minutes. The samples were then analysed using Coulter counter. Table 3A shows the results obtained with a suspension of 3.1×10^8 microbubbles/ml having a mean diameter of 2.1 µm.

Table 3A

Homogeneity of microbubble suspensions in the syringe as a function of the rotation rate and time (microbubble concentration 3.1×10^8 bubbles/ml)

Syringe			rotation			rates					
rpm	0	1.3	2	60	0	1.3	2	60	0	1.3	2	60
Vr	0	114	176	5278	0	114	176	5278	0	114	276	5278
t(min)	Nb total (%)				Nb>8µ (%)				Volume (%)			
0	100	100	100	100	100	100	100	100	100	100	100	100
5	68.7	77.6	90.4	97.4	23.5	48.0	73.3	95.4	37.5	55.6	80.4	97.7
10	53.7	77.3	88.8	100.6	1.1	43.9	70.9	98.9	19.8	44.8	73.3	99.4
15	48.2	72.8	89.5	96.2	1.9	38.0	74.1	96.5	14.5	44.0	75.7	98.1
20	43.5	73.8	86.6	99.0	0.8	37.2	77.9	97.3	10.8	42.5	73.6	98.6
25	39.9	76.4	88.5	100.3	0.5	36.9	84.6	99.5	9.6	43.0	81.6	99.7

Nb total (%) : percentage of the total bubble concentration as compared to value at t=0.

Nb>8µ(%) : percentage of the bubbles above 8µm as compared to value at t=0.

Volume (%) : percentage of total bubble volume per ml of solution as compared to value at t=0

rpm: rotation per minute; Vr (mm/min)=tangential speed of the syringe (radius=14mm)

Gas microbubbles: air/C_4F_{10} (50:50).

The above results clearly indicate that even at very low rotation rates (1.3 and 2 rpm), the buoyancy rise of the microbubbles is prevented. This is because even at low rotation rates, the tangential velocity of the microbubble is far greater than that of buoyancy. As previously shown, microbubbles of 3 and 10 µm have the respective rising rates of 0.29 and 3.3 mm/min. At 1.3 rpm rotation, the tangential speed is 114 mm/min (Vr = 2π x rpm x $R_{syringe}$) which makes the tangential component of the 3 µm microbubble 390 times greater than the buoyancy. For 10 µm

microbubble the tangential component is 35 times greater than the ascension rate. It should be mentioned that at very high rotation rates (e.g. 1,000 rpm) the microbubbles will concentrate in the middle of the syringe (as the radial component becomes dominant). Rotational speeds at which the radial component becomes important are not of interest as under such conditions the suspension becomes non-homogeneous again. The rotational speed at which the radial force is becoming significant depends on the syringe size (diameter, size of microbubbles and viscosity of the suspension) hence the exact value of the rotational speed at which the radial component becomes important is to be established for each individual case. However, as already pointed out such rotational speeds are to be avoided.

Table 3B

Homogeneity of microbubble suspensions in the syringe as a function of the rotation rate and time (microbubble concentration 1.3×10^9 bubbles/ml)

/	Syringe			rota	tion		rates					
rpm	0	3	12	60	0	3	12	60	0	3	12	60
Vr	0	264	1056	5278	0	264	1056	5278	0	264	1056	5278
t(min)	Nb	total	(%)		Nb>	8μ	(%)			Volume		(%)
0	100	100	100	100	100	100	100	100	100	100	100	100
5	6.0	26.0	76.8	81.3	0.5	16.0	73.1	83.4	1.5	17.2	81.4	87.7
10	3.2	26.3	78.8	81.3	0.2	19.1	71.5	79.9	1.0	20.0	81.9	79.4
15	3.9	27.3	81.5	82.2	0.6	16.8	78.0	80.5	1.1	20.3	84.9	90.8
20	4.3	32.0	76.6	95.0	0.2	19.2	79.6	85.9	1.8	21.5	92.3	92.6
25	0	31.7	78.9.	95.3	0	16.9	78.6	85.5	0	18.4	83.4	91.1

Nb total (%) : percentage of the total bubble concentration as compared to value at t=0.

Nb>8μ(%) : percentage of the bubbles above 8μm as compared to value at t=0.

Volume (%) : percentage of total bubble volume per ml of solution as compared to value at t=0

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rpm: rotation per minute; Vr (mm/min)=tangential speed of the syringe (radius=14mm)

Gas microbubbles: air/C_4F_{10}

5   For more concentrated suspensions (e.g. 1.3 x 10^9 bubbles/ml) the microbubble ascension rate increases in the syringe. This is probably due to microbubble interactions (associations, dragg etc.) indicating that higher rotation speeds are required for prevention of microbubble ascension in the suspensions with higher microbubble concentrations. However, the lower limit of the syringe rotation is not easy to determine as the microbubble ascention rate is also a function of viscosity and density of the suspension, the nature of the gas used, the microbubble diameter and size distribution as well as the type of the microparticles (i.e. microbubbles having just a layer of a surfactant stabilising the gas, microballoon with a tangible membrane or microemulsion).

Example 4

Evaluation of the efficiency of the rotary pump

A. Infusion of gas microbubble suspensions at low bubble concentration and "fast" infusion rate (3.3ml/min)

In this study, the phospholipid stabilised gas microbubbles were prepared with a gas mixture (air/perfluorobutane 50 : 50) as gas phase.

The efficacy of the rotary pump of the present invention was evaluated by checking the homogeneity and stability of the bubble suspensions during the infusion. During a continuous infusion, the bubble suspensions were successively sampled at different infusion times with an interval of about every 5 minutes. The syringe used for infusion had a effective volume of 60 ml with a diameter of 28 mm (Braun Perfusor, Germany). The rotation rate of the syringe was fixed at 60 rpm or 1 Hz (in order to compare different suspensions) and the direction of rotation was reversed for each turn. Infusion was stopped after 15

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minutes while maintaining syringe rotation and then restored at the same rate during one minute after 30 minutes and 60 minutes. The bubble concentration, sizes and size distribution were assessed with Coulter® Multisizer II

5   and the echo contrast effect of the suspensions was simultaneously examined with an echographic imaging device (Acuson 128XP10, USA). For Coulter® and echo evaluation, the native samples taken from the syringe were further diluted 1000 and 3000 folds (in some experiences 1/750).

10 For *in vitro* imaging evaluation, an acoustic phantom ATS (Peripheral Vascular Doppler Flow Phantom, ATS Laboratoies Inc., USA) was used and the image was visualised in B-mode with a 3.5 MHz ultrasound probe. The acoustic energy was set to minimum (-9dB) in order to prevent bubble

15 destruction. The results are summarised in the Table 4.

Table 4

Evaluation of the efficacy of the rotary pump
(Coulter and Echo imaging)

20 Gas microbubbles: air/C_4F_{10}

Pump flow rate : 3.3 ml/min

	Coulter measurement				Imaging
t(min)	Nbtot/mlx10^8	Nb>8µ/mlx10^6	Diam (µm)	Vol/ml	VI (pixels)
0	**3.26**	**4.90**	**2.09**	**7.33**	**62**
2	2.97	4.90	2.15	7.12	59
8	3.31	4.50	2.06	7.15	59
15	3.06	3.55	2.09	5.82	59
30	3.12	4.82	2.15	6.96	59
60	3.15	4.66	2.10	7.03	59

Nb>8µ/ml : number of bubbles above 8µm.

Nb total/ml : total bubble concentration.

25 Volume (µl/ml) :total bubble volume per ml of solution

Diameter (µm) :mean diameter in number.

VI : video intensity(dilution 1:3000)

These results show that even at a small rotation rate (1 Hz), the bubble suspension was fairly stable and homogeneous: both the total bubble count and bubbles>8μm remain constant during the entire infusion.

B. Infusion of gas microbubble suspensions at high bubble concentration and "slow" infusion rate (1.2 ml/min)

The example A was repeated at a "slow" infusion rate and a higher concentration of the microbubbles. One can note from the Table 5 that even at very slow infusion rate (corresponding to 0.017 ml/kg/min for a 70 kg person) and a very high bubble concentration (Nb/ml > 10^9/ml), the present rotary infusion pump is capable to ensure the stability and the imaging performance of the bubble suspensions during the entire infusion (24 min).

Table 5

Evaluation of the efficacy of the rotary pump (Coulter and Echo imaging)

Gas microbubbles: air/C$_4$F$_{10}$

Pump flow rate : 1.2 ml/min

	Coulter measurement				Imaging
t(min)	Nb/ml x 10^9	Nb>8μ/mlx10^7	Diameter (μm)	Volume/ml	VI (pixels)
0	**1.10**	**2.2**	**2.09**	**32.5**	**60**
5	1.03	2.2	2.15	30.9	60
13	1.01	2.1	2.06	30.5	55
18	1.03	2.1	2.09	30.0	58
24	1.04	2.1	2.15	30.5	57

Nb>8μ/ml : number of bubbles above 8μm.

Nb total/ml : total bubble concentration.

Volume (μl/ml) :total bubble volume per ml of solution

Diameter (μm) :mean diameter in number.

VI : video intensity(dilution 1:3000)

Example 5

<u>Evaluation of the efficacy of the rotary pump - comparative tests with and without syringe rotation</u>

The procedure of Example 4 was repeated except that the phospholipid stabilised microbubbles were prepared
5 with gas SF_6 instead of air/C_4F_{10}. Moreover, the stability of the gas bubble suspensions during infusion was compared using the same pump in the presence and absence of rotation of the syringe (R=28 mm, rotation rate = 60 rpm and 0 rpm). The experimental results of a concentrated bubble
10 suspension (Nb> 10^9/ml) infused at an infusion rate of 1.1 ml/min are shown in Table 6. Without syringe rotation, the amount of microbubbles delivered from the syringe decrease rapidly during infusion, especially for the large bubbles (see Nb>8μm and the volume). After 5 minutes of infusion,
15 the total bubble concentration decreased by 83%, >99% for the bubbles larger than 8μm and 90% for the bubble volume. After 10 minutes, the video intensity had decreased by a factor 3 and the contrast effect of the microbubbles was almost non detectable (IV = 6 ±3 pixels for the background)
20 at 10 minutes of the infusion. In contrast, in the presence of rotation the bubble suspension remained stable during the entire infusion (30 min).

Table 6

Evaluation of the efficacy of the rotary pump :
25 comparative test

Gas microbubbles: SF_6

Pump flow rate : 1.1 ml/min

	Coulter measurement								Imaging	
t(min)	Nb/ml	x 10^9	Nb>8μ	/mlx10^7	Diameter (μm)		Volume	/ml	VI	(pixels)
rot	with	w/out	with	w/out	with	w/out	with	w/out	with	w/out
0	**1.2**	**1.1**	**1.58**	**1.32**	**2.09**	**2.22**	**24.3**	**20.4**	**32**	**54**
5	1.16	0.19	1.41	0.008	2.09	2.14	22.6	2.0	30	16
10	1.06	0.15	1.37	0.012	2.13	1.96	21.4	1.2	30	10
15	1.15	0.13	1.38	0.00	2.11	1.92	22.5	0.9	30	8
20	1.38	0.12	1.81	0.00	2.13	1.83	28.5	0.7	31	7
30	1.25	0.11	1.53	0.00	2.11	1.79	24.4	0.6	32	6

Nb>8μ/ml : number of bubbles above 8μm.

Nb total/ml : total bubble concentration.

Volume (μl/ml) :total bubble volume per ml of solution

Diameter (μm) :mean diameter in number.

VI : video intensity(dilution 1:3000)

Table 7

Evaluation of the efficacy of the rotary pump : comparative tests

Gas microbubbles: SF_6

Pump flow rate : 3.3 ml/min

	Coulter		measurements							
t(min)	Nb tot	/mlx10^6	Nb>8μ	/ml x10^6	Diam.	(μm)	Vol.	/ml	Vol	%
rotat	with	w/out	with	w/out	with	w/out	with	w/out	with	w/out
0	2.73	2.48	3.14	2.99	2.22	2.09	5.5	4.9	100.0	89.4
5	2.57	2.05	3.21	0.5.4	2.23	1.94	5.3	2.1	96.7	39.1
10	2.53	1.71	3.42	0.01	2.27	1.81	5.8	1.1	105.7	20.6
15	2.48	1.41	3.28	0.00	2.28	1.64	5.2	0.6	96.1	10.8
20	2.35	1.16	1.33	0.00	2.21	1.54	4.6	0.5	83.6	8.3

Nb>8μ/ml : number of bubbles above 8μm.

Nb total/ml : total bubble concentration.

Volume (μl/ml) :total bubble volume per ml of solution

Diameter (μm) :mean diameter in number.

VI : video intensity(dilution 1:3000)

In Table 7, the comparative infusion was conducted at a lower bubble concentration (2.7 10^8/ml) and an infusion rate of 3.3 ml/min. Again, these results show a very good efficacy of the rotary infusion system to maintain the homogeneity and stability of the microbubble suspensions during infusion. In contrast, the syringe pump without rotation was completely inadequate for microbubble infusion.

Example 6

Evaluation of the efficiency of the rotary pump

21

<u>Application to gas microspheres (comparative tests)</u>

The Example 5 was repeated with the gas albumin microspheres prepared as described in Example 2. For the infusion, the bubble concentration was adjusted to 6×10^8/ml
5   by diluting the suspension with HSA/dextrose (1:3). In the present experience, the in vitro characteristics of the microspheres during infusion (2.7 ml/min) with and without the syringe rotation were compared to assess the homogeneity of the suspensions delivered from the syringe.
10 The results are gathered in Table 8.

Table 8

Evaluation of the efficacy of the rotary infusion pump with gas albumin microspheres
15   Gas microbubbles: C_3F_8

Pump flow rate : 2.7 ml/min

	Coult er		measurements							
t(min)	Nbtot	/mlx10⁸	Nb>8µ	/mlx10⁶	Diam	(µm)	Vol	/ml	VI	(pixels)
rot	with	w/out	with	w/out	with	w/out	with	w/out	with	w/out
0	6.64	6.7	9.6	7.6	2.03	1.97	15.4	12.3	47	46
5	6.4	6.6	8.0	4.5	1.96	1.93	13.1	8.8	47	38
10	6.4	6.3	6.0	2.6	1.91	1.8	10.3	5.0	45	23
15	6.4	6.0	6.5	0.38	1.92	1.65	10.5	3.5	45	19
20	6.25	5.2	6.15	0.15	1.92	1.61	9.9	3.8	43	26

Nb>8µ/ml : number of bubbles above 8µm.

Nb total/ml : total bubble concentration.
20   Volume (µl/ml) :total bubble volume per ml of solution

Diameter (µm) :mean diameter in number.

VI : video intensity(dilution 1:3000)

Background : VI = 9 pixels

25   The albumin microspheres appear to be more homogeneous in the syringe than phospholipid microbubbles in the absence of rotation. This is likely to be due to the higher viscosity of the albumin/dextrose solution (5%) and possibly to the thicker wall of the microspheres (about 15
30 times thicker than a phospholipid monolayer). Nevertheless,

large microspheres (>8µm) still decanted in the syringe and
their concentration decreased progressively during
infusion. After 10 minutes, the volume of microspheres and
the video intensity decreased to half of the initial
5   values. It was been reported that the myocardial perfusion
with a similar agent - FSO69 (Optison®, HSA-C_3F_8
microsphere suspensions) was attributed essentially to a
small number of large microspheres (10-15 µm) entrapped in
the tissue (Skyba et al., *J. Am. Coll. Cardio.* **28** (1996)
10   1292-1300). Therefore, on can speculate that for such
clinical application this kind of contrast agents could
hardly be infused by a classic infusion pump as
demonstrated in the present example.

15   Example 7

         Tetracaine filled tripalmitin microcapsules made
according to Example 6 of WO96/15815 were suspended in 50
ml of saline solution (0.9% NaCl). The suspension with a
20   concentration of tetracaine of 0.06 mg/ml was placed into a
50 ml syringe. The syringe was placed on the rotational
pump of the invention and the exit concentration of
tetracaine measured using UV spectrophotometer (in
THF/water 60/40% at 307 nm). The syringe was rotated at a
25   rate of 1Hz (alternating direction of rotation every 180°).
The rate of infusion was 1.5 ml/min. The UV analysis showed
constant concentration of tetracaine over the entire period
of infusion. In the parallel experiment in which the
tetracaine filled syringe was kept stationary the exit
30   concentration of the medicament varied with time.

         Example 8

         Fifty milligrams of Amphotericin B in the
35   deoxycholate form (Fungizone® Bristol Mayers Squibb) were
dispersed in 50 ml of Intralipid® 20% (Pharmacia) and the
emulsion obtained (Chavanet, P,. *Rev. Med. Interne* **18**
(1997) 153-165) introduced into a 50 ml syringe. The

syringe was placed on the rotational pump and infused at 1
ml/min rate and rotation of 1 Hz (alternating direction of
rotation every 360°. Exit concentration of Amphotericin B
was followed by HPLC (detection UV/visible at 405 nm). The
5   HPLC analysis confirmed constant concentration of the
medicament during the entire infusion. The experiment
clearly showed that the separation of Amphotericin B
reported by several research groups (Trissel, L. A., *Am. J.
Health Syst. Pharm.* **52** (1995) 1463; Owens, D., *Am. J.*
10   *Health Syst. Pharm.* **54** (1997) 683) was successfully
suppressed using the method disclosed.

Example 9

15   A liposome solution was prepared from 9/1 molar
ratio of hydrogenated soy lecithin (DPPC) and
dipalmitoylphosphatidic acid disodium salt (DPPA) in
chloroform according to a well known procedure (e.g. EP 0
514 523). After extrusion and cooling of MLV suspension the
20   same was concentrated to 30 mg/ml by microfiltration. To 1l
of the concentrated liposome solution, 1 l of an aqueous
solution containing 1040 g of (S)-N,N'-bis [2-hydroxy-1-
(hydroxymethyl)-ethyl]-2,4,6-triiodo-5-lactamido-
isophtalamide   (Iopamidol®, an X-ray contrast agent of
25   BRACCO S.p.A.) was added and the resulting mixture having
an iodine concentration of 260 g/l was incubated. The
density of the Iopamidol® solution was 1.29 g/cm^3.
            An aliquot of the liposome preparation was
dialyzed against saline (NaCl 0.9% in water) until all
30   iopamidol outside the liposomes vesicles was removed. The
iodine-to-lipid ratio of the preparation obtained (I/L) was
between 3 and 5 mg of entrapped iodine per mg lipid.
            Part of the preparation of contrast agent-loaded
liposomes was introduced into a syringe which was placed on
35   the rotational pump of the invention and the exit
concentration of the contrast agent measured using HPLC.
The syringe was rotated at a rate of 1Hz (alternating
direction of rotation every 180°). The rate of infusion was

1.5 ml/min. The HPLC analysis showed constant concentration of the iodinated contrast agent over the entire period of infusion.

When, in the foregoing example, the Iopamidol was replaced by Iomeprol (N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-5-glycolamido-isophtal-imide), another iodinated contrast agent from BRACCO S.p.A., similar results were experienced.

Claims

1. A method of administering to patients by
injection or infusion a suspension of microparticles
homogeneously distributed in an aqueous liquid carrier by
means of an injector system comprising a syringe containing
said suspension and a power driven piston for injecting
said suspension into a patient, characterised by subjecting
the suspension in the syringe to a rotation or rocking
motion, thereby maintaining said suspension homogeneous by
preventing segregation of the microparticles by gravity or
buoyancy, this being without damaging said particles or
disturbing their distribution.

2. The method of claim 1, in which said motion is
provided by outside means for imparting motion to said
particles, which motion is then transferred to said liquid
carrier.

3. The method of claim 1, in which said motion of
rocking or rotation is alternated.

4. The method of claim 3, in which said motion is
applied along or around the syringe longitudinal or
transverse axis.

5. The method of claim 4, in which said motion is
provided by subjecting the syringe to continuous or
intermittent rotation.

6. The method of claim 5, in which the rotation
rate is from 0.5 to 200 rpm.

7. The method of anyone of preceding claims, in
which said motion is alternating rotation the direction of
which is reversed every 30°, 60°, 90°, 180°, 270° or 360°.

8. The method of claim 7, in which the direction is alternated at a frequency of 0.5 Hz, 1.0, Hz, 1.5 Hz, 2.0 Hz, 2.5 Hz, 3.0 Hz or 3.5 Hz.

9. The method of anyone of preceding claims, in which said motion is carried out stepwise.

10. An injector system for administering to patients by injection or infusion a suspension of microparticles in an aqueous liquid carrier, said system comprising a syringe (22) whose barrel (25) contains said suspension, and automatic electromechanical power means (24, 31, 34) controllably acting on the syringe to inject the suspension into a patient, characterised in that said injector system further comprises means (30a, 30b) for agitating said microparticles in said suspension, said agitation keeping said suspension homogenous by preventing segregation of said particles by gravity or buoyancy without damaging said particles or disturbing their distribution.

11. The injector system of claim 10, in which said means (30a, 30b) for agitating the suspension in the syringe constitute means under motion for supporting the syringe in the system, the effect of said motion applied to the syringe being to agitate the liquid in the syringe barrel.

12. The injector system of claim 11, in which said motion is a rotation.

13. The injector system of claim 10 in which said injection means acting on the syringe include a syringe plunger (26) driven into forward or backward motion by helical screw means (23, 31, 32).

14. The injector system of claim 13, in which the position of the plunger in the syringe is governed by a

number of turns of said helical screw means as controlled by said automatic power means (31, 34).

15. The injector system of claim 12, characterized in that said means under rotation are constituted by wheels (13) in in contact with the syringe barrel for driving it into consecutive rotation.

16. The injector system of claim 12, in which the syringe rotates alternatively in one and the opposite direction.

17. The injector system of claim 16, in which the angle covered in each alternate rotation is 30°, 60°, 90°, 180°, 270° or 360°.

18. The injector system of claim 11, in which the rotation rate of the syringe is from 0.5 to 200 rpm.

19. The injector system of claim 11, further comprising a fixed laser detector for reading identification marks provided on the syringe.

20. The injector system of claim 10, further comprising safety means for interrupting the injector operation in case of emergency.

21 The injector system of claim 20, in which said security means operate by monitoring the force applied to the syringe during injection, a sudden increase of that force producing a signal for stopping the injector operation.

22. The injector system of anyone of claims 10-21, in which the suspension is a contrast agent for ultrasonic imaging of patients.

28

23. The injector system of claim 22, in which the contrast agent comprises, in suspension in an aqueous liquid carrier, gas filled microvesicles which are either microbubbles bounded by a gas/liquid interface made from
5   dissolved surfactants, or microballoons bounded by a material envelope made of organic polymers, or of di- or tri- glycerides.

24. The injector system of claim 23, in which the gas
10   is a pure physiologically acceptable halogenated gas or gas mixture comprising at least one physiologically acceptable halogenated gas.

25. The injector system of claim 24, in which the
15   halogenated gas is selected from CF_4, C_2F_6, C_3F_8, C_4F_8, C_4F_{10}, C_5F_{12}, C_6F_{14} or SF_6.

26. The injector system of claim 24, wherein the gas mixture contains a gas selected from air, oxygen, nitrogen,
20   helium, xenon or carbon dioxide.

27. The injector system of anyone of claims 23-26, in which at least one of the surfactants is a saturated phospholipid in a lamellar or laminar form.
25

28. The injector system of claim 27, in which at least one of the phospholipids is a diacylphosphatidyl compound wherein the acyl group is a C_{16} fatty acid residue or a higher homologue thereof.
30

29. The injector system of claim 23, in which the polymer of the membrane is selected from polylactic or polyglycolic acid and their copolymers, denatured serum albumin, denatured haemoglobin, polycyanoacrylate, and
35   esters of polyglutamic and polyaspartic acids.

29

30. The injector system of claim 29, in which the microballoons are filled with C_3F_8 and the material envelope is made from albumin.

5   31. The injector system of claim 23, in which the microballoons are bounded by saturated triglycerides, preferably tristearine, tripalmitine or mixtures of thereof with other glycerides, fatty acids and biodegradable polymers.

10

32. The injector system of anyone of claims 10-31, in which the suspension is a contrast agent for CT imaging.

33. The injector system of claim 32, in which the
15 contrast agent comprises as a suspension in a liquid carrier phase liposomes filled with an iodinated compound selected from iomeprol, iopamidol, iopentol, iohexol, metrizamide, iopromide, iogulamide, iosimide or ioversol.

20   34. The injector system of claim 33, in which iodine over lipid ratio I/L is 3 or more.

35. Use of the injector system according to anyone of claims 10-34 in imaging of organs, blood vessels and
25 tissues of a mammalian.

36. Use of claim 35, in which the imaging is ultrasonic imaging and the organ is the heart, the brain, the kidneys, the liver.

30

37. Use of the injector system according to anyone of claims 10-34 in imaging of organs, blood vessels and tissue of a mammalian.

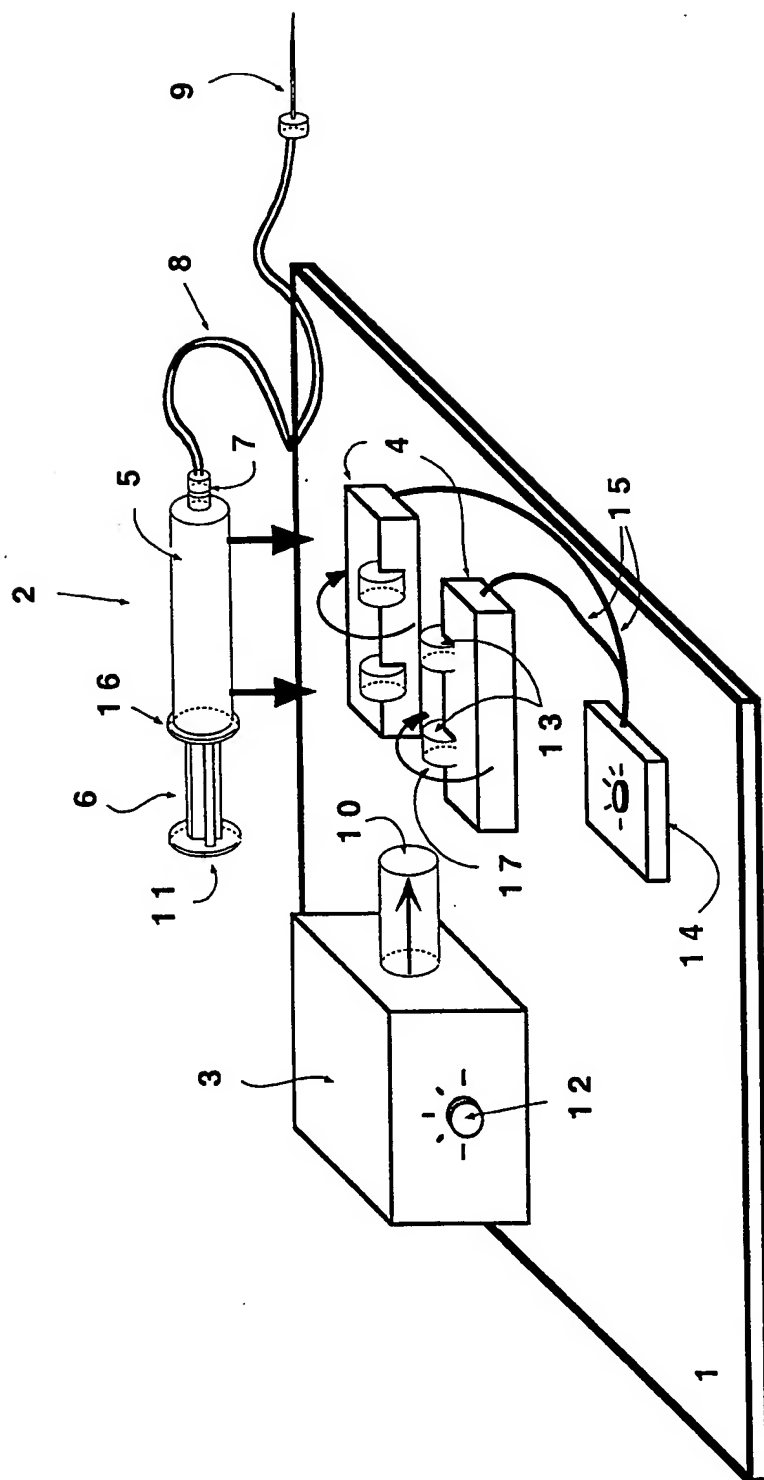35   38. Use according to claim 37, in which the imaging is CT imaging and the organ is the liver.

Fig. 1
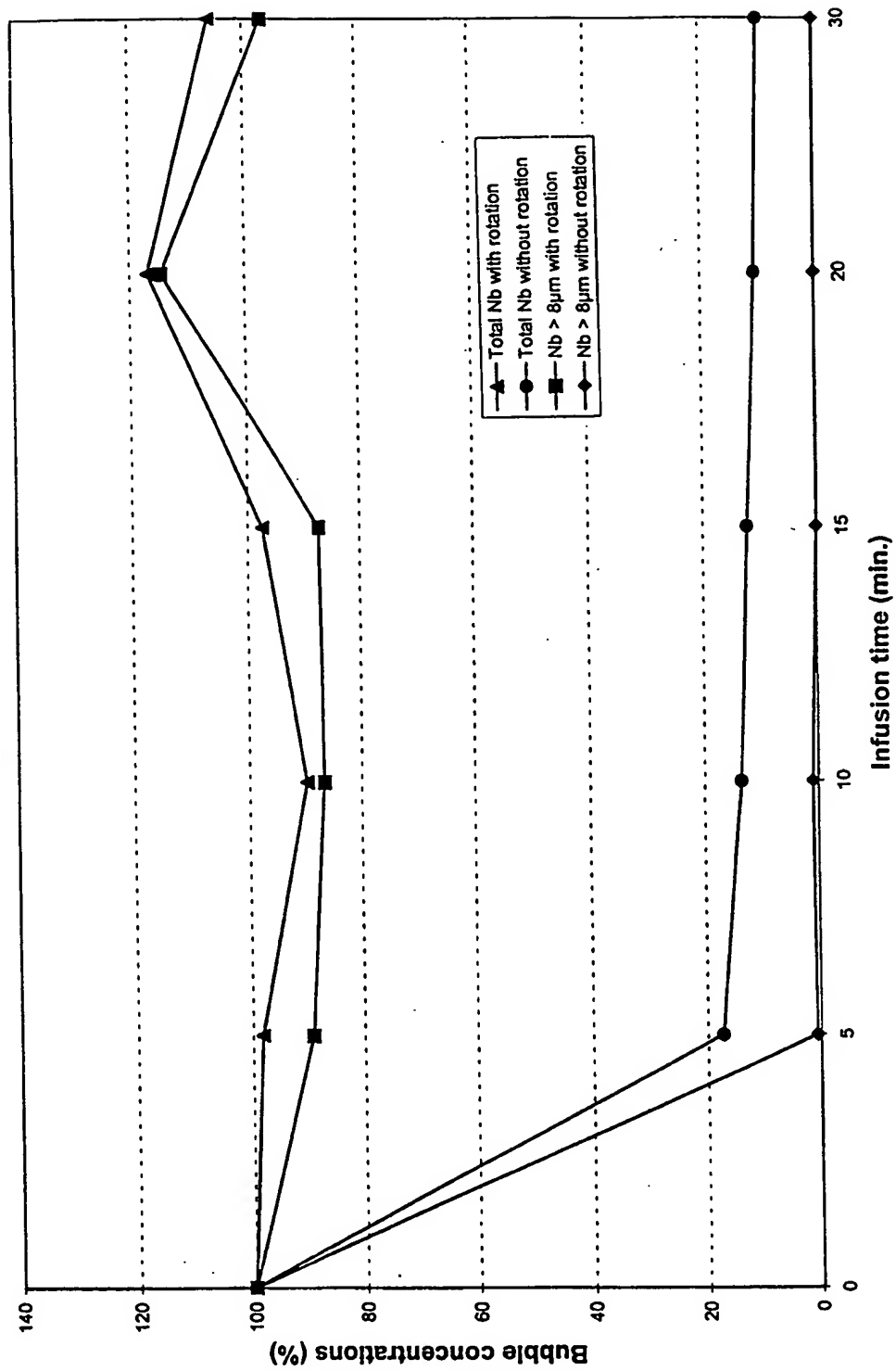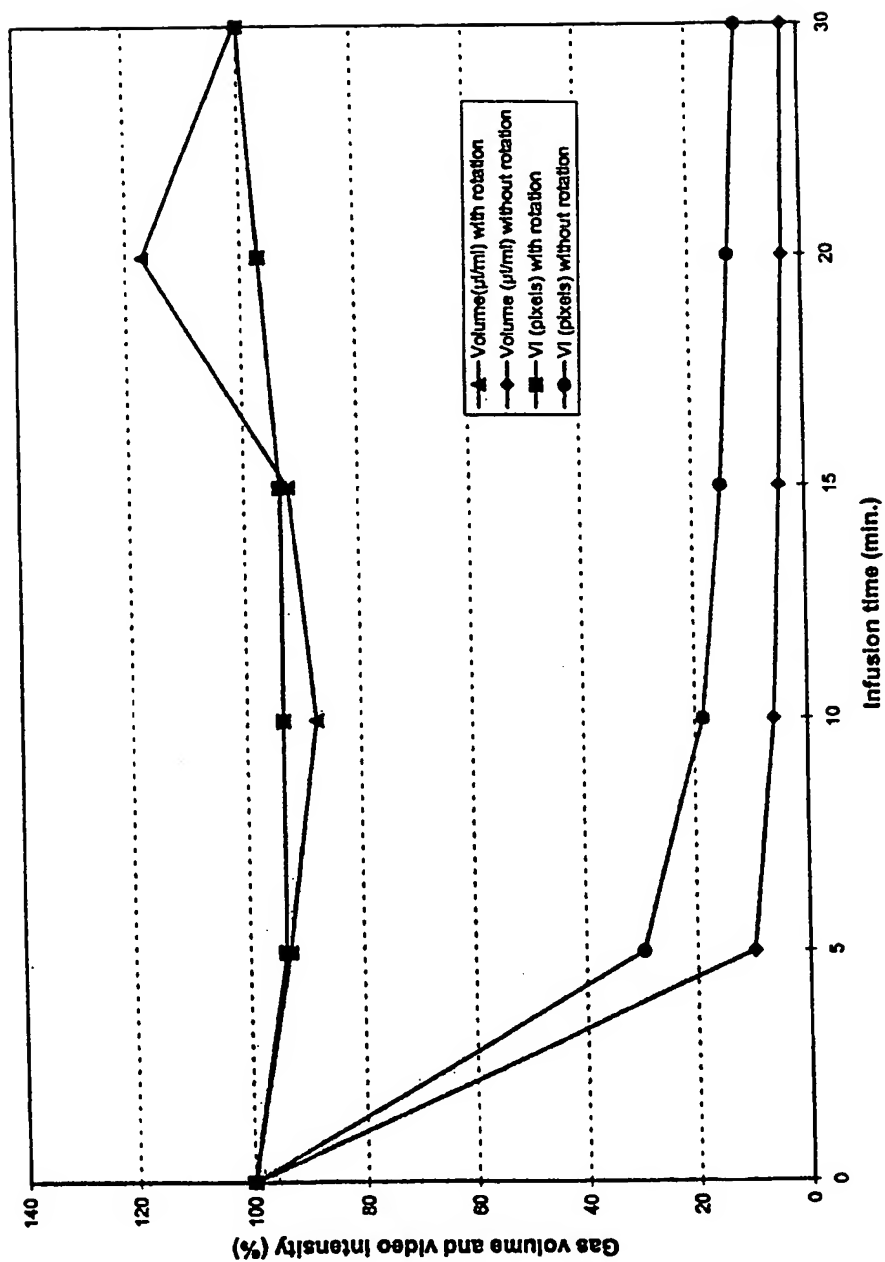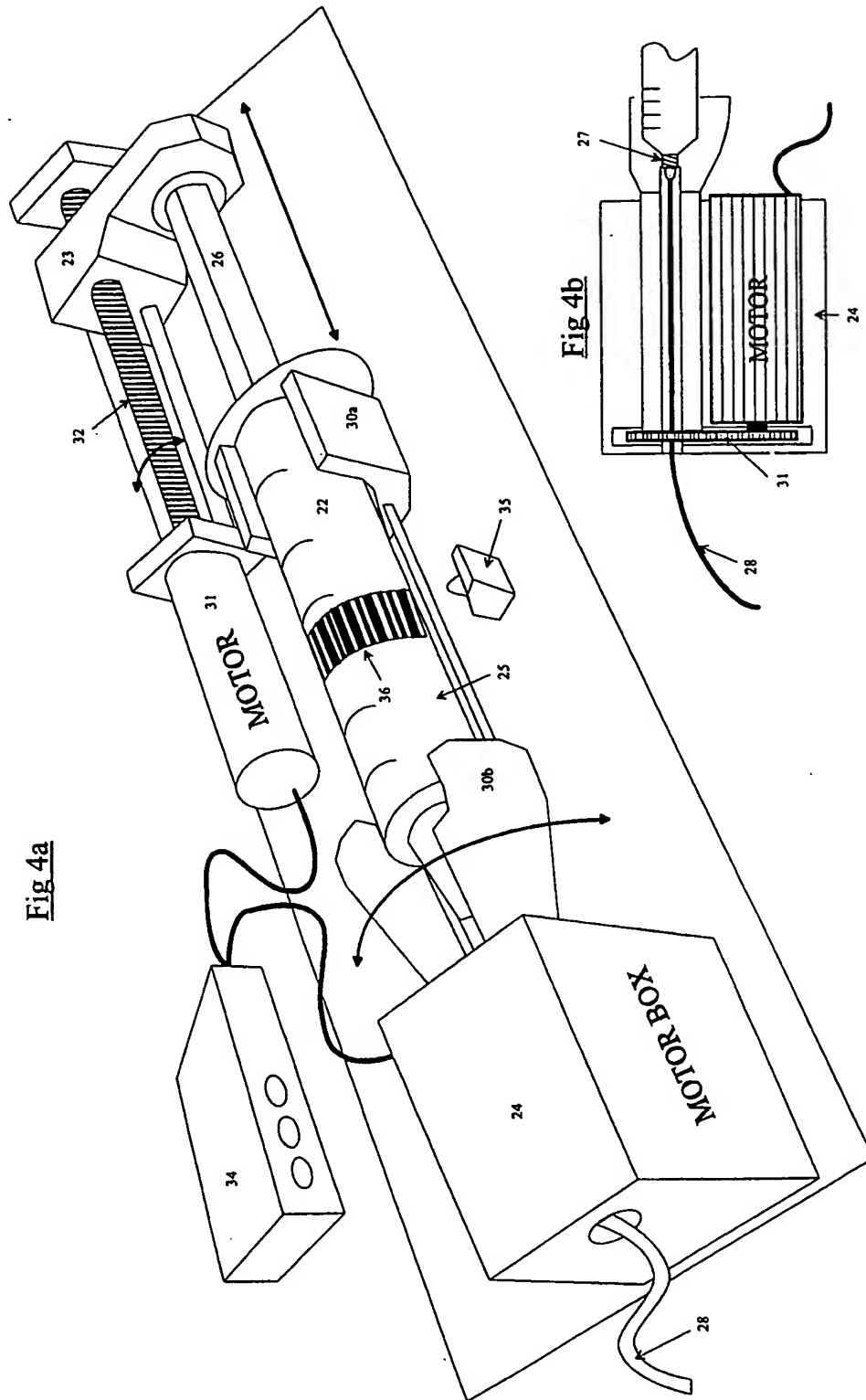
Fig. 2

Fig. 3

Fig 4a

Fig 4b

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   A61M5/145

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   A61M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 18420 A (BRACCO RESEARCH S.A.) 20 June 1996 see page 5, line 10 - line 17 ---	10
Y	FR 2 429 600 A (HYCO & AULAS ETS) 25 January 1980 see page 2, line 14 - line 26; claim 2; figure 1 ---	10
A	OWENS D. ET AL: "Stability of amphotericin B 0.05 and 0.5 mg/mL in 20% fat emulsion" AM. J. HEALTH-SYST. PHARMACISTS, vol. 54, 15 March 1997, pages 683-686, XP002064707 cited in the application see page 685, right-hand column, paragraph 2 ---	10

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| X | Further documents are listed in the continuation of box C. | | X | Patent family members are listed in annex. |

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the International search	Date of mailing of the international search report
9 April 1999	20/05/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Sedy, R

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate. of the relevant passages	Relevant to claim No.
A	US 5 401 253 A (REYNOLDS) 28 March 1995 see column 3, line 7 - line 12 ---	10
A	US 5 528 923 A (LEDEZ ET AL.) 25 June 1996 see column 18, line 57 - line 58 see column 19, line 55 - line 58 ---	10,11
A	US 5 686 060 A (SCHNEIDER ET AL.) 11 November 1997 see column 10, line 16 - line 18 see column 12, line 33 - line 36 see column 12, line 53 - line 57 ---	10,27
A	FR 2 220 228 A (METAC S.A.) 4 October 1974 see page 1, line 17 - line 22 ---	11
A	US 5 611 344 A (BERNSTEIN ET AL.) 18 March 1997 see column 3, line 33 - line 34 see column 6, line 24 - line 31 see column 6, line 54 - line 64 see column 8, line 45 - line 47 see column 8, line 66 - line 67 -----	22-26, 31,36,38

1

INTERNATIONAL SEARCH REPORT

Box I   Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:   1-9
   because they relate to subject matter not required to be searched by this Authority. namely:

   Rule 39.1(iv) PCT - Method for treatment of the human or animal body by surgery

2. [ ] Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II   Observations where unity of Invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application. as follows:

1. [ ] As all required additional search fees were timely paid by the applicant. this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee. this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant. this International Search Report covers only those claims for which fees were paid. specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently. this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9618420	A	20-06-1996	AU	701807 B	04-02-1999
			AU	3935295 A	03-07-1996
			CA	2181340 A	20-06-1996
			CN	1141001 A	22-01-1997
			CZ	9602420 A	13-11-1996
			EP	0744962 A	04-12-1996
			FI	963202 A	15-08-1996
			JP	9509433 T	22-09-1997
			NO	963327 A	09-08-1996
			NZ	295744 A	22-08-1997
			US	5827504 A	27-10-1998
			US	5830435 A	03-11-1998
			ZA	9510721 A	20-06-1996
FR 2429600	A	25-01-1980	NONE		
US 5401253	A	28-03-1995	GB	2274064 A	13-07-1994
US 5528923	A	25-06-1996	CA	2112004 A	22-06-1994
US 5686060	A	11-11-1997	AT	146972 T	15-01-1997
			AU	2618197 A	18-12-1997
			AU	681812 B	04-09-1997
			AU	3431795 A	04-01-1996
			AU	666238 B	01-02-1996
			AU	5336294 A	24-05-1994
			CA	2125027 A,C	11-05-1994
			CN	1088456 A	29-06-1994
			DE	69307124 D	13-02-1997
			DE	69307124 T	17-07-1997
			DK	619743 T	20-01-1997
			WO	9409829 A	11-05-1994
			EP	0619743 A	19-10-1994
			ES	2097548 T	01-04-1997
			FI	943167 A	01-07-1994
			GR	3022826 T	30-06-1997
			HU	74561 A	28-01-1997
			IL	107453 A	04-01-1998
			JP	7503254 T	06-04-1995
			NO	942476 A	30-06-1994
			NZ	257115 A	24-06-1997
			NZ	280615 A	24-02-1997
			US	5445813 A	29-08-1995
			US	5597549 A	28-01-1997
			ZA	9308117 A	23-06-1994
FR 2220228	A	04-10-1974	NONE		
US 5611344	A	18-03-1997	AU	2139297 A	22-09-1997
			CA	2247151 A	12-09-1997
			CZ	9802812 A	13-01-1999
			EP	0904113 A	31-03-1999
			NO	984089 A	04-09-1998
			NZ	331460 A	23-12-1998
			PL	328690 A	15-02-1999
			WO	9732609 A	12-09-1997
			US	5853698 A	29-12-1998